1: Acta Virol 1999 Dec;43(6):367-72 Related Articles, Books, LinkOut

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Unintegrated viral DNA as a marker for human immunodeficiency virus 1 infection in vivo and in vitro.

Nandi JS.

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Related Resources

The unintegrated viral DNA found in human immunodeficiency virus (HIV) infection includes linear and circular forms. The circular unintegrated viral DNA (CUVD) could be of either 1-long terminal repeat (LTR) or 2-LTR form. Inverse primers from nef (upstream) and gag (downstream) gene sequences of HIV-1 genome were designed to span the LTR circle junction. CUVD was assayed in unstimulated, quiescent persistently infected cell lines 8E5, HIIIB, and GB8, as well as in peripheral blood lymphocytes (PBLs) of HIV-1-infected patients by nested PCR in a cross sectional study. CUVD in the infected cell lines (in vitro) was predominantly of 2-LTR form in 8E5 and GB8 cells, while in HIIIB cells, there was besides 1-LTR and 2-LTR an additional, intermediate form. In vivo, CUVD was predominantly of 1-LTR form. The possibility of using CUVD, an early phenomenon in the virus replication, as an additional postpenetration, preintegration marker of HIV infection is discussed.

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ANSWER 9 OF 44 BIOSIS COPYRIGHT 2001 BIOSIS
AN 1995:149142 BIOSIS
DN PREV199598163442
TI ***HIV*** -1 acquires resistance to AZT and delavirdine in vitro by multiple RT substitutions.
AU Slade, D. E. (1); Dueweke, T. J. (1); Poppe, S. M. (1); Swaney, S. M. (1); Wisniewski, S. M. (1); Sharova, V.; ***Stevenson, M.*** ; Tarpley, W. G. (1)
CS (1) Upjohn Lab., Kalamazoo, MI USA
SO AMERICAN SOCIETY FOR MICROBIOLOGY.. (1995) pp. 89. Human retroviruses and related infections.
Publisher: American Society for Microbiology (ASM) Books Division, 1325 Massachusetts Ave. NW, Washington, DC 20005-4171, USA.
Meeting Info.: 2nd National Conference Washington, D.C., USA January 29-February 2, 1995
ISBN: 1-55581-097-7.
DT Conference
LA Englis

L2 ANSWER 13 OF 44 BIOSIS COPYRIGHT 2001 BIOSIS
AN 1994:169981 BIOSIS
DN PREV199497182981
TI Reduced nuclear import of human immunodeficiency virus type 1 preintegration complexes in the presence of a prototypic nuclear targeting signal.
AU Gulizia, J.; Dempsey, M. P.; Sharova, N.; Bukrinsky, M. I.; Spitz, L.; Goldfarb, D.; ***Stevenson, M. (1)***
CS (1) Depd. Pathol. Microbiol, Univ. Nebraska Med. Cent., 600 S. 42nd St., Omaha, NE 68198-5120 USA
SO Journal of Virology, (1994) Vol. 68, No. 3, pp. 2021-2025.
ISSN: 0022-538X.
DT Article
LA English

ANSWER 18 OF 44 BIOSIS COPYRIGHT 2001 BIOSIS
AN 1992:447058 BIOSIS
DN BR43:80058
TI FEATURES GOVERNING NUCLEAR IMPORT OF ***HIV*** -1 PREINTEGRATION COMPLEXES ROLE IN PERMISSIVENESS LATENCY AND REACTIVATION.
AU ***STEVENSON M*** ; BUKRINSKY M I; SHAROVA N; GULIZIA J; HAGGERTY S
CS UNIV. NEBR. MED. CENT., 600 SOUTH 42ND ST., OMAHA, NEBR. 68198-5120.
SO VIII INTERNATIONAL CONFERENCE ON AIDS AND THE III STD WORLD CONGRESS. VIII INTERNATIONAL CONFERENCE ON AIDS AND THE III STD WORLD CONGRESS; HARVARD-AMSTERDAM CONFERENCE, AMSTERDAM, NETHERLANDS, JULY 19-24, 1992. PAGINATION VARIES VIII INTERNATIONAL CONFERENCE ON AIDS AND THE III STD WORLD CONGRESS: AMSTERDAM, NETHERLANDS. PAPER. (1992) 0 (0), TH71.
DT Conference
FS BR; OLD

integrase protein, and the defect could therefore be caused by an inactive integrase. An amino acid neutral mutant containing four purine-to-pyrimidine changes in the PPT showed delayed replication as well as a lower production of gapped DNA molecules.

Conclusion: The coincindence of delayed replication and lower frequency of gapped viral DNA in the purine-to-pyrimidine mutant is consistent with the notion that the function of the central PPT is to provide a second starting point for plus-strand synthesis, and that this function is important for virus replication. The use of two starting points may enable the virus to complete its DNA synthesis more rapidly, or increase the probability of successful reverse transcription and, consequently, infection.

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ThA 1535

FEATURES GOVERNING NUCLEAR IMPORT OF HIV-1 PREINTEGRATION COMPLEXES: ROLE IN PERMISSIVENESS, LATENCY AND REACTIVATION. Stevenson, Mario; Bukrinsky, MI; Sharova, N*; Gulizia, J; and Haggerty, S. University of Nebraska Medical Center, Omaha, NE, USA; *D. I. Ivanovsky Institute of Virology, Moscow, Russia

The cytopathogenic properties of HIV-1 in permissive CD4 lymphocytes in vitro are difficult to reconcile with the chronic nature of AIDS progression and the gradual decline in CD4 lymphocyte number. Thus, my laboratory has investigated features of the virus and the host which influence HIV-1 latency and reactivation during disease progression. Our published observations (EMBO J 9:1551, 1990; Science 254:423, 1991) demonstrate that quiescent T lymphocytes are a major virus reservoir in HIV-1 infected individuals. Infection of quiescent lymphocytes is nonproductive due to an unidentified block to HIV-1 integration, however, subsequent T-cell activation promotes renewed DNA integration and virus production.

We have extended our observations to identify the block to HIV-1 integration in quiescent T cells. Analysis of the distribution of viral DNA in quiescent and activated T cells isolated from HIV-1 infected individuals indicates that viral DNA in quiescent lymphocytes is exclusively cytoplasmic and these cells do not support nuclear import of viral DNA. More detailed analysis has revealed that the preintegration complex of HIV-1 is transported to the nucleus of the host cell in a process which is independent of cell division but which requires ATP: features which are indicative of an active transport process. We have begun characterizing components of the preintegration complex which govern its active nuclear import. Our studies suggest that the matrix antigen (MA) of HIV-1 is a component of the preintegration complex of HIV-1. By virtue of a nuclear localization signal (NLS) at the N terminus of MA, this antigen is important for nuclear import of the viral preintegration complex. Mutations within this NLS restrict nuclear import of HIV-1 DNA following virus infection. In addition, peptide analogues of the NLS of MA specifically block HIV-1 replication in permissive CD4 cells in vitro due to their ability to restrict nuclear import of HIV-1 DNA.

These studies identify critical early events in the life cycle of HIV-1 and their dependence on host cell processes. The presence of an active transport pathway for nuclear import of HIV-1 preintegration complexes may provide insight into mechanisms governing viral latency. In addition, the ability to interrupt nuclear import of HIV-1 DNA represents a novel strategy for the interruption of HIV-1 replication.

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ThA 1531

THREE-DIMENSIONAL STRUCTURE OF HIV-1 REVERSE TRANSCRIPTASE COMPLEXED WITH A dsDNA TEMPLATE-PRIMER REVEALS ARRANGEMENT OF THE ACTIVE SITES FOR POLYMERIZATION AND RNase H. Arnold, Edward*; Jacobo-Molina, A.*; Nanni, R.G.*; Williams, R.L.*; Clark, A.D., Jr.*; Ding, J.*; Lu, X.*; Ferris, A.L.**; Clark, P.K.**; Hughes, S.H.** *Center for Advanced Biotechnology and Medicine (CABM) and Rutgers University, Piscataway, NJ, **National Cancer Institute-Frederick Cancer Research and Development Center.

Objectives: Knowledge of the three-dimensional structure of HIV reverse transcriptase (RT) in a catalytically relevant complex should potentially enable improved design of RT inhibitors. Molecular mechanisms of catalysis and drug resistance should also be illuminated through studies of the structure.

Methods: We are applying a combination of X-ray crystallography and molecular biology to the problem of obtaining a structure of HIV-1 RT in atomic detail. We have obtained crystals of HIV-1 RT complexed with a 19/18 one-base overhang dsDNA template-primer mimic and a monoclonal antibody Fab fragment that diffract X-rays to 3.1 Å resolution at the Cornell High Energy Synchrotron Source.

Results: We have determined the 7 Å resolution structure of a ternary complex of HIV-1 RT p66/p51 heterodimer, a monoclonal antibody Fab fragment, and a 19/18 dsDNA. The dsDNA is well ordered and binds in a groove on the surface of the enzyme. Near one end of the electron density corresponding to dsDNA, the arrangement of the electron density matches well with the known structure of a polypeptide corresponding to the HIV-1 RT RNase H. At the opposite end of the dsDNA, a mercurated derivative of uridine triphosphate has been localized by difference Fourier methods, allowing tentative identification of the polymerase nucleoside triphosphate addition site. We have also independently determined the structure of the RT:Fab complex in the absence of DNA at 7 Å resolution, permitting comparison of the bound and free forms of the enzyme.

Conclusions: These results and the ongoing higher resolution structure determination have important implications for understanding polymerase interactions with nucleic acid substrates and the development of improved RT inhibitors for the treatment of AIDS.

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ThA 1532

THE SECOND ORIGIN OF HIV DNA PLUS-STRAND : STRUCTURE AND ROLE IN VIRAL REPLICATION.
Charneau, P., and Clavel, F.
Unité d'oncologie virale, Institut Pasteur, Paris.

OBJECTIVES : Most retroviruses have a unique, well defined origin site for their DNA plus-strand, determined by a polypurine tract (PPT). By contrast, HIV and some related viruses use an additional origin site, located at the center of the viral genome, and defined by a second copy of the PPT, which result in a discontinuity (gap) in linear DNA plus-strand. We wished to better define the role of this structure in HIV replication.

METHODS AND RESULTS : We created mutations replacing purines by pyrimidines in the HIV-1 central PPT, which left intact the overlapping amino-acid sequence. We found that these mutations are able to significantly slow down viral growth; The delay in growth kinetics is proportionnal to the number of introduced pyrimidines and to the decrease in the amount of discontinuous (gapped) linear molecules in cells infected with the corresponding mutants. The PPT mutants also display a proportionnal reduction of infectious titer when assayed on HeLa-CD4 cells following a single round of HIV replication, which suggests that the central PPT is not only able to improve the speed of viral DNA synthesis, but also increases the overall efficiency of reverse transcription. The introduction of a new copy of the PPT at a different site in a mutant lacking a normal central PPT creates a new plus-strand origin at that site. We could show that the presence of the stable "gap" in HIV DNA is due to a stop in elongation of upstream viral DNA plus-strand some 100 nucleotides past the origin of the downstream segment at the central PPT. This limited strand displacement appears to be an intrinsic property of reverse transcriptase. This finding could affect our understanding of the mechanism of LTR duplication during retroviral DNA synthesis, which has been proposed to involve strand displacement events.

CONCLUSIONS : Our findings demonstrate the importance of a second plus strand origin site for efficient HIV replication, and further establish the role of PPTs as such initiation sites.

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Tel : 33/1/45 68 89 02. Fax : 33/1/45 68 88 85.

ThA 1533

THE CENTRAL POLYPURINE TRACT IN THE HIV-1 GENOME IS IMPORTANT FOR VIRAL REPLICATION. Grinde, Bjørn; Jensrud, K.; Tjøtta, E.; Hungnes, O. National Institute of Public Health, Oslo, Norway

Objectives: To understand the function of the central polypurine tract.

Introduction: The reverse transcription of HIV-1 RNA generates a linear, double-stranded DNA with a single-stranded gap. The gap is believed to be the result of plus-strand priming from a second, centrally located polypurine tract (PPT). This PPT is an exact duplication of the PPT at the border of the downstream LTR, which acts as the primer for plus-strand DNA synthesis in all retroviruses.

Results: The biological role of the central PPT was investigated by site-directed mutagenesis. Products from primer-directed PCR mutagenesis were cloned into an infectious molecular HIV-1 clone. Virus expressed in COS-1 cells was used to infect the human T-cell line MT4. We were unable to detect rplication of a mutant with the PPT deleted. This mutation also causes the loss of five amino acids from the integrase protein, and the defect could therefore be caused by an inactive integrase. An amino acid neutral mutant containing four purine-to-pyrimidine changes in the PPT showed delayed replication as well as a lower production of gapped DNA molecules.

Conclusion: The coincindence of delayed replication and lower frequency of gapped viral DNA in the purine-to-pyrimidine mutant is consistent with the notion that the function of the central PPT is to provide a second starting point for plus-strand synthesis, and that this function is important for virus replication. The use of two starting points may enable the virus to complete its DNA synthesis more rapidly, or increase the probability of successful reverse transcription and, consequently, infection.

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ThA 1534

PREFERENCE FOR G->A HYPERMUTATION VIA DISLOCATION MUTAGENESIS BY THE HIV-1 REVERSE TRANSCRIPTASE
Vartanian, Jean-Pierre, Andreas Meyerhans, Monica Sala, Henri Buc*, Michel Henry and Simon Wain-Hobson
Laboratoire de Rétrovirologie Moléculaire and Unité de Physiochimie des Macromolecules*, Institut Pasteur, Paris

Objectives: To characterize in vitro the mechanism of G->A hypermutation by dislocation mutagenesis and to compare the ability of the HIV-1, Moloney MLV and AMV reverse transcriptases (RTase) to tolerate dislocations.
Methods: A 60-mer DNA oligonucleotide was constructed so that both +1 and +2 frameshifts could be detected using the blue/white β-galactosidase assay. Experiments were performed with the HIV-1, MoMLV and AMV RTases as well as Klenow enzyme for different elongation times in the absence of dCTP. After which an excess of dCTP was added to complete the reaction. The final product was PCR amplified and cloned into M13mp18. White recombinants were screeded with oligonucleotide probes specific for both the +1 and +2 frameshifts. Positives were confirmed by sequencing.
Results: More than 30K colonies were screened. The HIV-1 RTase resulted in a greater number of +1 frameshifts than either the MoMLV or AMV enzymes. +2 frameshifts were never found.
Discussion and conclusion: These data show that G->A hypermutation, via dislocation mutagenesis, may occur more frequently during DNA synthesis when the HIV-1 enzyme is used as opposed to the MoMLV or AMV RTases.

ThA 1535

FEATURES GOVERNING NUCLEAR IMPORT OF HIV-1 PREINTEGRATION COMPLEXES: ROLE IN PERMISSIVENESS, LATENCY AND REACTIVATION. Stevenson, Mario; Bukrinsky, M.I.; Sharova, N.*; Culisia, J.; and Haggerty, S. University of Nebraska Medical Center, Omaha, NE, USA; *D. I. Ivanovsky Institute of Virology, Moscow, Russia

The cytopathogenic properties of HIV-1 in permissive CD4 lymphocytes in vitro are difficult to reconcile with the chronic nature of AIDS progression and the gradual decline in CD4 lymphocyte number. Thus, my laboratory has investigated features of the virus and the host which influence HIV-1 latency and reactivation during disease progression. Our published observations (EMBO J 9:1551, 1990; Science 254:423, 1991) demonstrate that quiescent T lymphocytes are a major virus reservoir in HIV-1 infected individuals. Infection of quiescent lymphocytes is nonproductive due to an unidentified block to HIV-1 integration, however, subsequent T-cell activation promotes renewed DNA integration and virus production.

We have extended our observations to identify the block to HIV-1 integration in quiescent T cells. Analysis of the distribution of viral DNA in quiescent and activated T cells isolated from HIV-1 infected individuals indicates that viral DNA in quiescent lymphocytes is exclusively cytoplasmic and these cells do not support nuclear import of viral DNA. More detailed analysis has revealed that the preintegration complex of HIV-1 is transported to the nucleus of the host cell in a process which is independent of cell division but which requires ATP: features which are indicative of an active transport process. We have begun characterizing components of the preintegration complex which govern its active nuclear import. Our studies suggest that the matrix antigen (MA) of HIV-1 is a component of the preintegration complex of HIV-1. By virtue of a nuclear localization signal (NLS) at the N terminus of MA; this antigen is important for nuclear import of the viral preintegration complex. Mutations within this NLS restrict nuclear import of HIV-1 DNA following virus infection. In addition, peptide analogues of the NLS of MA specifically block HIV-1 replication in permissive CD4 cells in vitro due to their ability to restrict nuclear import of HIV-1 DNA.

These studies identify critical early events in the life cycle of HIV-1 and their dependence on host cell processes. The presence of an active transport pathway for nuclear import of HIV-1 preintegration complexes may provide insight into mechanisms governing viral latency. In addition, the ability to interrupt nuclear import of HIV-1 DNA represents a novel strategy for the interruption of HIV-1 replication.

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ThA 1536

INFLUENCE OF HIV INFECTION ON THE Vβ REPERTOIRE IN MONOZYGOTIC TWINS DISCORDANT FOR HIV: EVIDENCE FOR THE EFFECTS OF A SUPERANTIGEN.
Pantaleo, Giuseppe*; Rebai, N.**; Graziosi*, C.; Lane, H.C.*; Sekaly, R.P.*; Fauci, A.S.*
*LIR, NIAID, NIH, Bethesda, MD, USA, **Lab. Immunology, IRCM, Montreal, Canada.

Objectives: To investigate whether perturbations of the Vβ repertoire occur in individuals infected with HIV and to determine whether these perturbations are consistent with the effects of a superantigen.
Methods: A quantitative polymerase chain rection (PCR) assay was used to determine the frequency of expression of 35 Vβ segments encompassing the 24 known Vβ families in a series of 9 HIV+ HLA mismatched individuals; 3 pairs of HIV+ monozygotic twins; 4 HIV+, HLA mismatched individuals; 6 pairs of monozygotic twins one of whom is infected with HIV and one of whom is not.
Results: Analysis of the Vβ repertoire in HIV+ monozygotic twins and in HIV+ HLA mismatched individuals clearly demonstrated the profound influence of the HLA complex on the expressed T cell receptor (TCR) Vβ repertoire. The expressed Vβ repertoire was very similar between HIV+ monozygotic twins, whereas a high degree of variability in the expressed Vβ repertoire was found among HIV+ HLA mismatched individuals. Analysis of the Vβ repertoire in 4 HIV+ HLA mismatched individuals at different stages of disease showed as much variability as that noted among HIV+, HLA mismatched individuals. Based on these results, it was difficult to determine whether these differences were related to a deletion event mediated by HIV infection or caused by intrinsic differences in the HLA haplotype of these individuals. To circumvent this problem, we analyzed the expressed Vβ repertoire by quantitative PCR in six pairs of discordant monozygotic twins one of whom was infected with HIV. Comparative analysis of the Vβ repertoire in each pair of twins showed that a limited set of distinct and partially overlapping Vβs were perturbed (i.e. expanded or deleted). Vβ 1 and Vβ 21 were perturbed in 3 of 6 HIV+ patients, while Vβ 16, 17 and 19 were perturbed in 2 of 6 individuals tested.
Conclusions: These results further confirm the importance of HLA matching in the analysis of the TCR in normal or pathological conditions. Furthermore, they are consistent with the effects of a superantigen in HIV infection, and based on murine models of retroviral infections, a superantigen stimulation may represent a fundamental mechanism responsible, at least in part, for the quantitative and qualitative abnormalities of CD4+ T lymphocytes observed in HIV infected individuals.

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Ulrike Winkler
09/478,170
812
1648

LA English

2 ANSWER 17 OF 44 BIOSIS COPYRIGHT 2001 BIOSIS
AN 1992:447350 BIOSIS
DN BR43:80350
TI COMPONENTS OF ***HIV*** -1 PREINTEGRATION COMPLEX NUCLEAR IMPORT IS ASSOCIATED WITH COMPLEX MATURATION.
AU BUKRINSKY M; SHAROVA N; PUSHKARSKAYA T; SHAPIRO I; ***STEVENSON M***
CS UNIV. NEBR. MED. CENT., 600 SOUTH 42ND ST., OMAHA, NEBR. 68198-5120.
SO VIII INTERNATIONAL CONFERENCE ON AIDS AND THE III STD WORLD CONGRESS. VIII INTERNATIONAL CONFERENCE ON AIDS AND THE III STD WORLD CONGRESS; HARVARD-AMSTERDAM CONFERENCE, AMSTERDAM, NETHERLANDS, JULY 19-24, 1992. PAGINATION VARIES VIII INTERNATIONAL CONFERENCE ON AIDS AND THE III STD WORLD CONGRESS: AMSTERDAM, NETHERLANDS. PAPER. (1992) 0 (0), A45.
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ANSWER 22 OF 44 BIOSIS COPYRIGHT 2001 BIOSIS
AN 1992:318727 BIOSIS
DN BR43:19452
TI NUCLEAR TRANSPORT OF ***HIV*** -1 GENETIC MATERIAL IMPLICATIONS FOR LATENCY AND PATHOGENESIS.
AU BUKRINSKY M I; SHAROVA N K; ***STEVENSON M***
CS DEP. PATHOLOGY MICROBIOLOGY, UNIVERSITY NEBRASKA MEDICAL CENTER, OMAHA, NEBR. 68198-5120.
SO KEYSTONE SYMPOSIUM ON PREVENTION AND TREATMENT OF AIDS, KEYSTONE, COLORADO, USA, MARCH 27-APRIL 3, 1992. J CELL BIOCHEM SUPPL. (1992) 0 (16 PART E), 46.
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DT Conference
FS BR; OLD
LA English

ANSWER 2 OF 5 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
AN 97172861 EMBASE
DN 1997172861
TI Evaluation of the presence of 2-LTR HIV-1 unintegrated DNA as a simple molecular predictor of disease progression.
AU Zazzi M.; Romano L.; Catucci M.; Venturi G.; De Milito A.; Almi P.; Gonnelli A.; Rubino M.; Occhini U.; Valensin P.E.
CS M. Zazzi, Sezione di Microbiologia, Dipartimento di Biologia Moleculare, Universita di Siena, Via Laterina 8, 53100 Siena, Italy
SO Journal of Medical Virology, (1997) 52/1 (20-25).
Refs: 28
ISSN: 0146-6615 CODEN: JMVIDB
CY United States
DT Journal; Article
FS 004 Microbiology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LA English

L1 ANSWER 1 OF 5 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
AN 1998106029 EMBASE
TI Unintegrated ***circular*** ***HIV*** -1 DNA in the peripheral mononuclear cells of HIV-1-infected subjects: Association with high levels of plasma HIV-1 RNA, rapid decline in CD4 count, and clinical progression to AIDS.
AU Panther L.A.; Coombs R.W.; Zeh J.E.; Collier A.C.; Corey L.
CS L.A. Panther, Harvard Medical School, West Roxbury Veterans' Admin. Hosp., Mailcode 11A, 1400 V.F.W. Parkway, West Roxbury, MA 02132, United States
SO Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology, (1998) 17/4 (303-313). ✓
Refs: 62
ISSN: 1077-9450 CODEN: JDSRET
CY United States
DT Journal; Article
FS 004 Microbiology

PoA 2252

COMPONENTS OF HIV-1 PREINTEGRATION COMPLEX: NUCLEAR IMPORT IS ASSOCIATED WITH COMPLEX MATURATION.

Bukrinsky, Michael*; Sharova, N.*; Dempsey, M.*; Stanwick, T.*; Bukrinskaya, A.*; Haggerty, S.*; Stevenson, M.* *Dept. of Pathology/Microbiology, Univ. of Nebraska Med. Ctr., Omaha, NE, USA •D.I. Ivanovski Inst. of Virology, Moscow, Russia, §Dept. of Immunology, Karolinska Inst., Stockholm, Sweden

Objectives: To characterize the components of HIV-1 preintegration complex and the mode of its maturation in an infected cell.

Methods: HIV-1 nucleoprotein complexes were purified from the nuclei and cytoplasm of infected MT-4 cells by non-detergent lysis, high salt extraction of nuclei and subsequent density equilibrium centrifugation in Nycodenz or immunoprecipitation with antisera to MA p17 or integrase. Fractions were analyzed by Western blot and by PCR specific for HIV-1 DNA and RNA. Functional activity was checked by *in vitro* integration assay.

Results: HIV-1 nucleoprotein complexes from cytoplasm and nuclei of infected MT-4 cells were found to peak at a density of 1.26-1.36 g/cm³ and demonstrated integration activity *in vitro*. Protein analysis revealed HIV-1 reverse transcriptase, integrase and matrix antigen (p17) as components of these complexes. HIV-1 DNA and RNA were both associated with the complex. RNAse treatment led to disintegration of the complex, leaving only HIV-1 DNA bound to integrase. AZT treatment blocked nuclear import of nucleoprotein complexes, indicating that DNA synthesis is a prerequisite for efficient nuclear import. However, reverse transcription appears to be completed predominantly in the nucleus, since agents inhibiting nuclear import (i.e. metabolic inhibitors) also inhibited completion of HIV-1 DNA synthesis. The presence of HIV-1 RNA and active reverse transcriptase in the nuclear preintegration complex supports the hypothesis that HIV-1 DNA synthesis and thus maturation of the preintegration complex are completed in the nucleus of the host cell.

Conclusion: HIV-1 preintegration complex was found to contain HIV-1 DNA, RNA, reverse transcriptase, integrase and MA p17. RNA is an important structural component of the complex, binding together p17, reverse transcriptase and HIV-1 DNA, while HIV-1 DNA seems to be tightly bound only to integrase. Nuclear import of this complex is dependent on the initiation of DNA synthesis, probably due to conformational changes associated with this process. Our results provide information on critical early events following HIV-1 infection: composition of preintegration complex, its maturation and mechanism of nuclear import. These studies provide the rationale for future attempts to interfere with early steps in HIV-1 replication that precede provirus establishment.

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PoA 2253

MUTANT HIV-1REVERSE TRANSCRIPTASE WITH ALTERED PROTEOLYTIC PROCESSING AND POLYMERASE ACTIVITY.

Laura Goobar Larsson et al. Dep. of Virology, Karolinska Institute, Stockholm, Sweden.

The conserved Asp-488 residue at the carboxy-terminal of HIV-1 reverse transcriptase was substituted by alanine and the mutant enzyme was expressed with or without HIV-1 proteinase in E. Coli. Purified mutant reverse transcriptase was characterized with respect to proteolytic processing and polymerase and RNase H activity. The point mutation alters the ratio of p66/p51 produced so that large amounts of p51 are obtained. It also increases the sensitivity of the heterodimer to proteolytic processing in vitro. The mutation does not cause any alteration of RNase H activity but it causes a reduction of polymerase activity. Km and Vmax for different substrates and IC50 values for different polymerase inhibitors were determined for the mutant enzyme and compared with wild-type values. Studies with monoclonal antibodies indicate alterations in the heterodimeric structure of reverse transcriptase as a consequence of this mutation.

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PoA 2254

NEW RT ASSAYS BASED ON CARRIER-BOUND TEMPLATES AND ^{3}H-LABELED dNTPs, USEFUL FOR ANALYSES OF THE MECHANISMS OF ACTION OF INHIBITORS AND FOR CHARACTERIZATION OF RT-MUTANTS.

Lennerstrand Johan, Neumüller M, Killander CFR, Gronowitz JS.
The Research Unit for Replication Enzymology, Uppsala, Sweden

Objectives: We have earlier documented the use of carrier-bound prA/odT in combination with ^{125}IdUTP for sensitive RT measurements (BAB 12:34-56, 1990, BAB 13:127-142, 1991). Following studies have shown the unique capacity of carrier-bound templates for simple evaluation of the mechanism of action of various RT inhibitors (in press Antivir.Chem. and Chemother. 3:1-7, 1992). The goal of the current study is to apply the new technique for other carrier-bound templates and for use with ^{3}H-labeled dNTPs. This in order to simplify the use of the new techniques wherever studies of RT-inhibitors or RT-mutants is required.

Methods: By the use of different coupling reactions either template prC or primer odG was bound covalently to polycarbonate or polystyrene beads. Thereafter, the primer or template amount optimal for assays discriminating between different steps in the RT-reaction was determined. The use of ^{3}H-labeled dNTP as substrate was evaluated by parallel RT-assays using the earlier system based on ^{125}IdTP and prA/odT-^{3}H-dGTP was applied for the prC/odG system. Both systems were then used in parallel to compare and document their use for RT-assays, analyses of RT inhibitors and studies of RT-mutants.

Results: The comparison of carrier-bound prA, prC, odT and odG showed that the three former gave similar sensitivity in the RT assay, while the last was hardly functional. The reduced sensitivity and higher variation in the values of double samples by use of ^{3}H do not hamper the assays for discrimination between RT-inhibition caused by chaintermination, by substrate competition, by non-enzymatic template destruction or by RT binding. This as well as the results with the original system will be exemplified using various known RT-inhibitors.

Conclusions: Carrierbound template/primer are efficient tools for RT-analyses and gives important information regarding to the mechanism of action of various potential antivirals and may thus indicate what cocktails give synergistic effects. Such cocktails may both decrease the risk for mutations leading to therapy resistance and may also be useful in concentrations giving low side-effects. Further, these constructs should be useful for identifying the different sites of RT involved in different steps of the RT-reaction when used with various RT-mutants.

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PoA 2255

INTERACTION OF HIV1-RT WITH AZIDOTHYMIDINE TRIPHOSPHATE AND THE NONNUCLEOSIDE INHIBITOR L-697,661.

Olsen, David B., Carroll, S.S., Bennett, C.D., Stern, A.M., Shafer, J.A., and Kuo, L.C. Merck Sharp & Dohme Research Laboratories, West Point, PA 19486, USA

The inhibition of HIV-1 reverse transcriptase (HIV-1 RT) by combinations of azidothymidine triphosphate (AZTTP) and the Merck compound L-697,661 has been examined. Cell-based assays showed synergistic inhibition of viral p24 production with combinations of AZT and L-697,661 (PNAS 88:6863). To determine if the apparent synergy results from direct inhibition of HIV-1 RT, reactions involving the incorporation of dTMP into poly*rA*-oligo*dT* were carried out. Synergistic inhibition of HIV-1 RT was observed only when the reaction was inhibited by more than 90%. This result is expected if the affinity of AZTTP for the L-697,661-bound HIV-1 RT complex (or conversely, the affinity of L-697,661 for the AZTTP-bound complex) is weak. Applying an empirically derived equation, an interaction coefficient, α, of 5.6 was determined.

The potency of inhibition of L-697,661 is greater with poly*rC*-oligo*dG* (IC_{50} = 20 nM) than with poly*rA*-oligo*dT* (IC_{50} = 830 nM), suggesting that the action of L-697,661 may be dependent on the sequence of the template. RNA sequences derived from the HIV-1 genome are being used as templates for synthesis by HIV-1 RT to study the sequence dependence of the potency of L-697,661. Results indicate that the potency of inhibition of synthesis on these heteromeric templates varies with the template primary structure, with the most potent inhibition being comparable to that observed on poly*rC*-oligo*dG*. The extent of inhibition at saturating concentrations of L-697,661 is less than 100% and dependent on template sequence.

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PoA 2256

SPONTANEOUS REVERSION IN A REVERSE TRANSCRIPTASE-DEFECTIVE INTEGRATED HIV GENOME.

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Unité d'Oncologie Virale, Institut Pasteur, Paris.

OBJECTIVES : To study the emergence of an infectious virus from a clonal cell line harboring a single, reverse transcriptase (RT)-defective copy of an integrated HIV-1 genome.

METHODS : The 8E5 cell line, derived from HIV-1-infected CEM cells, carries a single, RT-defective copy of an integrated HIV genome. The lack of RT production by this genome is the consequence of a frameshift in the pol gene, due to a single base addition at position 3241. These cells express gag and env proteins and produce HIV particles that have been described as non-infectious.

RESULTS : We recently found that cocultivation of 8E5 cells with MT4 or SupT1 cells resulted in the emergence of an infectious virus. This virus (termed 8E5R) is RT positive, but displays a slow replication profile, together with a reduced cytopathic effect, and can be serially passaged on CD4+ lymphoid cells. Nucleotide sequence of a segment of the 8E5R pol region produced by PCR amplification shows that the single nucleotide insertion characteristic of the 8E5 genome had been corrected, but that most of the base changes that can differentiate 8E5 from the HIV-1_{LAI} isolate, from which it was derived, were conserved. We are currently examining the possible mechanisms of the reversion, which could include frameshift read-through, transcriptional misreading, or complementation by another reverse transcriptase.

CONCLUSIONS : The observation of an apparently spontaneous correction of a mutation in a defective HIV genome could be important, in regard of the possible role of defective viral genomes in HIV infection.
In addition, the 8E5 cell line is used in many laboratories, notably as a standard for PCR quantification, and is generally considered as unable to produce infectious virus. Our findings should prompt investigators to use particular care in the handling of these cells.

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PoA 2257

Identification of residues on HIV-1 reverse transcriptase interacting with its cognate primer tRNA^{Lys3}

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§Boehringer Mannheim GmbH, Research Center Penzberg, F.R.G.

Objective: The replication of retroviral genomes is initiated by reverse transcriptase (RT) catalyzed elongation of a tRNA molecule bound at the primer binding site (PBS) adjacent to the U5 region of the viral RNA. In the case of HIV-1, human-tRNA^{Lys3} has been proposed to function as the primer for reverse transcription. In positioning its cognate tRNA to the PBS, RT itself plays an important role. This offers an interesting target for chemotherapeutic intervention. As a prerequisite for an approach towards this, we were interested in determining the residues of HIV-1 RT which are involved in this specific protein/nucleic acid interaction.

Methods: Purified radioactively labelled tRNA molecules (human-tRNA^{Lys3} or bovine-tRNA^{Lys3}) were used to study their interaction with recombinant heterodimeric HIV-1 RT (1) by a gel retardation assay (2). Using a set of 23 murine monoclonal antibodies (MAb) prepared against HIV-1 RT (3), we have investigated their effect on the observed tRNA/RT interaction. The monoclonal antibodies recognize residues within amino acids 200-230, 300-428 and 528-560 of the RT polypeptide.

Results and Discussion: Two antibodies were found to block the tRNA/RT interaction completely. These MAbs have been mapped to aa 300-350 and residues around aa 540 respectively. Two other antibodies, both interacting with residues 300-350, were shown to stimulate the observed tRNA/RT interaction. The remaining MAbs showed no effect.
In interpreting these results, we propose that aa 300-350 and residues around aa 540 are involved in the specific interaction of HIV-1 RT with its cognate primer tRNA^{Lys3}.

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(2) Weiss, S. et al., (1992) Gene, in press
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TRACK A: POSTER BASIC SCIENCE

PoA 2252

COMPONENTS OF HIV-1 PREINTEGRATION COMPLEX: NUCLEAR IMPORT IS ASSOCIATED WITH COMPLEX MATURATION.

Bukrinsky, Michael*; Sharova, N+; Pushkarskaya, T*; Shapiro, I§; Stevenson, M* *Dept. of Pathology/Microbiology, Univ. of Nebraska Med. Ctr., Omaha, NE, USA +D.I. Ivanovski Inst. of Virology, Moscow, Russia, §Dept. of Immunology, Karolinska Inst., Stockholm, Sweden

Objectives: To characterize the components of HIV-1 preintegration complex and the mode of its maturation in an infected cell.

Methods: HIV-1 nucleoprotein complexes were purified from the nuclei and cytoplasm of infected MT-4 cells by non-detergent lysis, high salt extraction of nuclei and subsequent density equilibrium centrifugation in Nycodenz or immunoprecipitation with antisera to MA p17 or integrase. Fractions were analyzed by Western blot and by PCR specific for HIV-1 DNA and RNA. Functional activity was checked by *in vitro* integration assay.

Results: HIV-1 nucleoprotein complexes from cytoplasm and nuclei of infected MT-4 cells were found to peak at a density of 1.26-1.36 g/cm^3 and demonstrated integration activity *in vitro*. Protein analysis revealed HIV-1 reverse transcriptase, integrase and matrix antigen (p17) as components of these complexes. HIV-1 DNA and RNA were both associated with the complex. RNAse treatment led to disintegration of the complex, leaving only HIV-1 DNA bound to integrase. AZT treatment blocked nuclear import of nucleoprotein complexes, indicating that DNA synthesis is a prerequisite for efficient nuclear import. However, reverse transcription appears to be completed predominantly in the nucleus, since agents inhibiting nuclear import (i.e. metabolic inhibitors) also inhibited completion of HIV-1 DNA synthesis. The presence of HIV-1 RNA and active reverse transcriptase in the nuclear preintegration complex supports the hypothesis that HIV-1 DNA synthesis and thus maturation of the preintegration complex are completed in the nucleus of the host cell.

Conclusion: HIV-1 preintegration complex was found to contain HIV-1 DNA, RNA, reverse transcriptase, integrase and MA p17. RNA is an important structural component of the complex, binding together p17, reverse transcriptase and HIV-1 DNA, while HIV-1 DNA seems to be tightly bound only to integrase. Nuclear import of this complex is dependent on the initiation of DNA synthesis, probably due to conformational changes associated with this process. Our results provide information on critical early events following HIV-1 infection: composition of preintegration complex, its maturation and mechanisms of nuclear import. These studies provide the rationale for future attempts to interfere with early steps in HIV-1 replication that precede provirus establishment.

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PoA 2254

NEW RT ASSAYS BASED ON CARRIER-BOUND TEMPLATES AND ^{3}H-LABELED dNTPs, USEFUL FOR ANALYSES OF THE MECHANISMS OF ACTION OF INHIBITORS AND FOR CHARACTERIZATION OF RT-MUTANTS.

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Objectives: We have earlier documented the use of carrier-bound prA/odT in combination with ^{125}IdUTP for sensitive RT measurements (BAB 12:34-56, 1990, BAB 13:127-142, 1991). Following studies have shown the unique capacity of carrier-bound templates for simple evaluation of the mechanism of action of various RT inhibitors (in press Antivir.Chem. and Chemother. 3:?-?, 1992). The goal of the current study is to apply the new technique for other carrier-bound templates and for use with ^{3}H-labeled dNTPs. This in order to simplify the use of the new techniques wherever studies of RT-inhibitors or RT-mutants is required.

Methods: By the use of different coupling reactions either template prC or primer odG was bound covalently to polycarbonate or polystyrene beads. Thereafter, the primer or template amount optimal for assays discriminating between different steps in the RT-reaction was determined. The use of ^{3}H-labeled dNTP as substrate was evaluated by parallel RT-assays using the earlier system based on ^{125}IUdTP and prA/odT-^{3}H-dGTP was applied for the prC/odG system. Both systems were then used in parallel to compare and document their use for RT-assays, analyses of RT inhibitors and studies of RT-mutants.

Results: The comparison of carrier-bound prA, prC, odT and odG showed that the three former gave similar sensitivity in the RT assay while the latter was only functional. The reduced sensitivity and higher variation in

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2 ANSWER 17 OF 44 BIOSIS COPYRIGHT 2001 BIOSIS
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TI COMPONENTS OF ***HIV*** -1 PREINTEGRATION COMPLEX NUCLEAR IMPORT IS ASSOCIATED WITH COMPLEX MATURATION.
AU BUKRINSKY M; SHAROVA N; PUSHKARSKAYA T; SHAPIRO I; ***STEVENSON M***
CS UNIV. NEBR. MED. CENT., 600 SOUTH 42ND ST., OMAHA, NEBR. 68198-5120.
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DT Conference
FS BR; OLD
LA English

ANSWER 22 OF 44 BIOSIS COPYRIGHT 2001 BIOSIS
AN 1992:318727 BIOSIS
DN BR43:19452
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AU BUKRINSKY M I; SHAROVA N K; ***STEVENSON M***
CS DEP. PATHOLOGY MICROBIOLOGY, UNIVERSITY NEBRASKA MEDICAL CENTER, OMAHA, NEBR. 68198-5120.
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CODEN: JCBSD7.
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FS BR; OLD
LA English

ANSWER 2 OF 5 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
AN 97172861 EMBASE
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L1 ANSWER 1 OF 5 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
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Q 306 NUCLEAR TRANSPORT OF HIV-1 GENETIC MATERIAL: IMPLICATIONS FOR LATENCY AND PATHOGENESIS, Michael I. Bukrinsky, Natalia K. Sharova, and Mario Stevenson, Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, Nebraska 68198-5120

We have recently demonstrated that quiescent T cells are an inducible reservoir of latent HIV-1 in asymptomatic individuals (Bukrinsky et al., Science, 254:423-427, 1991). HIV-1 DNA is preserved in unintegrated state in these cells, but it is capable of integration upon cell activation. Here we report that block to integration in quiescent T cells is caused by inefficient HIV-1 DNA transport into the nucleus. Using HIV-1 1-LTR and 2-LTR circular DNAs (detected by PCR) as an indicator of successful nuclear transport, we have shown that the preintegration complex of HIV-1 is rapidly transported into the nucleus of the host cell by a process which requires ATP but which is independent of the cell cycle. A functional integrase protein is not necessary for the active nuclear transport of HIV-1 preintegration complexes. In the nucleus the HIV-1 DNA is found in two peaks after equilibrium density centrifugation: one with density 1.46 g/ml and another 1.36 g/ml. Reverse transcriptase activity was associated with the second peak. We suppose that the first peak represents mature preintegration complexes, while the second peak contains immature preintegration complexes with incomplete species of HIV-1 DNA.

These findings indicate that HIV-1 reverse transcription may proceed in the nucleus, as described for other lentiviruses. The preintegration complexes enter the nucleus by an active ATP-dependent mechanism. However, the nuclear transport is independent of cell cycle. These data are pertinent to our understanding of the mode of HIV-1 replication, as well as infection of terminally differentiated cells such as macrophages, dendritic and microglial cells.

Q 307 SIV EXPRESSION IN THE SPINAL CORD IS LOCALIZED TO THE MACROPHAGE AND ASSOCIATED WITH A SPECIFIC PATHOLOGIC FINDING. H. Burger[1], P. Campbell[1,2], A. Lackner[1], D. LaNeve[2], N. Peress[2], and B. Weiser[1]. Wadsworth Center for Research, Albany, NY[1], SUNY Stony Brook, NY[2], New Mexico Regional Primate Research Lab, Las Cruces, NM[3].

To evaluate the SIV-infected macaque as a model for AIDS-related neurologic disease, we studied spinal cords from SIV-infected animals. Previously, we studied a characteristic spinal cord finding in human AIDS patients called vacuolar myelopathy. We established that: 1) HIV-1 RNA is expressed in spinal cords with vacuolar myelopathy but not in control cords; 2) HIV-1 expression is localized to the macrophage; and 3) the level of HIV-1 RNA expression is directly correlated with the severity of clinical and pathological disease.

To extend our analysis to SIV and characterize the SIV-infected macaque as a model for HIV-1 neuropathogenesis, we continued to study the spinal cord. By using *in situ* hybridization, we analyzed spinal cords from 4 SIV-infected macaques with giant cell myelitis, an entity in SIV-infected macaques histologically resembling HIV-1 encephalitis in humans. In all 4 cords, we found high level SIV RNA expression. SIV RNA was localized primarily to the giant cell lesions, and to a lesser extent, to infiltrating inflammatory cells. Double-label analysis using combined *in situ* hybridization-immunohistochemistry, as well as immunohistochemistry alone identified both the multinucleated giant cells and mononuclear inflammatory cells to be monocyte/-macrophage derived. As controls, we studied 9 infected animals with either normal spinal cords or myelitis due to documented opportunistic infection. The control cords showed minimal or undetectable levels of SIV expression, including 3 from animals with SAIDS who had CMV infection and macrophage infiltration of the spinal cord. These results parallel those in HIV-1 infection, where HIV-1 expression was detected only in cords with vacuolar myelopathy. They extend the previous studies demonstrating a role for immunodeficiency viruses in tissue pathogenesis and document in detail that the SIV-infected macaque is an excellent model to study the mechanisms of HIV-1 related neuropathogenesis *in vivo*.

Q 308 THE RELATIONSHIP BETWEEN HIV-1 VIRAL TITER AND VIRAL DNA COPY NUMBER IN PBMC OF CHILDREN WITH TRANSFUSION-ACQUIRED INFECTION. Chelyapov, N.V., Courville, T., Wittek, A.E., Brunell, P.A., Israele, V. Ahmanson Pediatric Center, Cedars-Sinai Medical Center, UCLA School of Medicine, Los Angeles, CA 90048.

We have previously shown that progression of HIV infection in children was associated with an increase in the HIV-1 viral burden, as demonstrated by endpoint dilution culture techniques (Pediatrics 1991;87:921). For the same cohort of patients, we determined the HIV-1 viral DNA copy number in PBMC by quantitative PCR using end-labeled primers in the LTR-gag region of the viral genome. The mean increase in HIV-1 DNA copy number from PBMC of P1 (asymptomatic) to P2 (symptomatic) patients was smaller than the mean increase in viral titer. Patients with HIV-1 titers of 5 TCID/10^6 PBMC had a mean DNA copy number of 401/10^6 PBMC. In those with an HIV titer of 500 TCID/10^6 PBMC, the mean DNA copy number was found to be 9314/10^6 PBMC. Thus, a 100-fold increase in infectious virus titers was associated with only a 23-fold increase in the DNA copy number. The DNA to TCID ratio decreased from 80 in patients having a virus titer of 5 TCID/10^6 PBMC to 18 in patients with a virus titer of 500 TCID/10^6 PBMC. All observed differences were statistically significant. Fractionation of DNA from infected PBMC into high and low molecular weight fractions showed a predominance of extrachromosomal viral DNA for P1 patients and integrated DNA for P2 patients. These preliminary results provide some insight into the apparently greater efficiency of proviral DNA as HIV-1 disease progresses.

Q 309 V3 SEQUENCE ANALYSIS OF HIV-1 ISOLATES FROM BLOOD AND CSF INDICATES MARKERS FOR DISEASE PROGRESSION BUT DOES NOT IDENTIFY TISSUE-SPECIFIC DETERMINANTS. Francesca Chiodi, Barbara Keys, Bengt Fadell, Jenny Karis. Department of Virology, Karolinska Institute, Stockholm, Sweden.

The possibility exists that HIV-1 isolates infecting the brain undergo a process of adaptation in the tissue which select neurotropic variants of the virus. The HIV-1 V3 loop has been shown to be an important determinant for cell tropism. Accordingly, we have molecularly characterized isolates obtained in parallel from blood and cerebrospinal fluid (CSF) of 4 asymptomatic carriers, 2 patients with lymphoadenopathy and 4 AIDS patients. The first passage in PBMC was used for amplification by PCR with nested oligonucleotide primers which hybridize to conserved sequences flanking the V3 domain. PCR products (798 bp) were cloned into PGEM4z vector and an avarage of 4 clones from each isolate were sequenced. The resulting amino acid (aa) sequence from each clone consisted of 34 aa from the N-terminal flank, 35 aa from the V3 loop and 32 aa from the C-terminal flank.

The aa sequences of the clones from each virus were used to generate a consensus aa sequence. Blood and CSF isolates were compared to one another and to a consensus of U.S./European sequences. Based on this approach, we could not find clear evidence for tissue-specific signature sequences. Two aa residues (Asn 289 and His 308) however, appear to correlate with progression from early to advanced stage of HIV-1 infection. Experiments designed to establish the replicative capacity of the CSF and blood isolates in primary monocytes, T- and monocytoid cell lines are in progress.

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AB We observed 36 HIV-infected patients to evaluate whether the presence of tandem 2-long terminal repeat circular unintegrated HIV-1 DNA (2-LTR) in peripheral blood mononuclear cells (PBMC) at baseline was associated with acceleration of HIV disease. Detection of 2-LTR at baseline correlated with high plasma HIV-1 RNA levels ($p < .01$), recovery of culturable HIV-1 from plasma ($p = .02$), and progression to AIDS during follow-up ($p = .01$).

More patients with 2-LTR (68%) than without 2-LTR (31%) had a decline in CD4 levels of >50 cells/mm3 over the first 18 months of follow-up ($p = .04$), and the average annual CD4 decline was 35% in patients with 2-LTR compared with 16% in those without 2-LTR ($p = 0.06$). Detection of 2-LTR in PBMC at baseline was an independent predictor of high plasma HIV-1 RNA levels and subsequent CD4 cell decline in this cohort of patients with predominantly non-syncytium- inducing (NSI) isolates at baseline. The presence of 2-LTR in PBMC appears to be reflective of ongoing HIV-1 replication, as measured by plasma HIV-1 RNA levels, and identifies persons

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AN 97172861 EMBASE

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CS M. Zazzi, Sezione di Microbiologia, Dipartimento di Biologia Moleculare, Universita di Siena, Via Laterina 8, 53100 Siena, Italy

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AB In a preliminary cross-sectional analysis of 109 human immunodeficiency virus type 1 (HIV-1) infected subjects the presence of 2-long terminal repeat (LTR) unintegrated **circular HIV**-1 DNA in peripheral blood mononuclear cells (PBMC) was found to be associated with both symptomatic infection (P = 0.0037) and low CD4 counts (P = 0.0004). To investigate the prognostic significance of the presence of 2-LTR HIV-1 DNA, a subset of 23 2-LTR-negative and 25 2-LTR-positive asymptomatic individuals were followed up for 12-24 months. The two groups did not differ in terms of baseline CD4 counts, zidovudine (ZDV) therapy, and duration of HIV-1 infection. Longitudinal analysis of CD4 values did not indicate a significantly different CD4 outcome between the two groups. However, when only ZDV-treated subjects were considered, a significant (P = 0.042) decrease in CD4 counts was found at month 24 with respect to baseline in 2-LTR-positive (n = 12) but not in 2-LTR-negative (n = 11) patients. Moreover, when >40% CD4 loss from baseline and/or development of CDC stage B or C symptoms were considered as indicators of disease progression, there was a significantly higher number of events in the whole 2-LTR-positive group than in the whole 2-LTR negative group (P = 0.0197 at month 12, P = 0.0299 at month 18, P = 0.0373 at month 24). Thus, the presence of 2-LTR HIV-1 DNA in PBMC merits further investigation as a simple, qualitative, molecular predictor of disease progression and decreased response to antiretroviral therapy.

1: AIDS Res Hum Retroviruses 1996 Mar 1;12(4):315-23

Detection of unintegrated HIV type 1 DNA in cell culture and clinical peripheral blood mononuclear cell samples: correlation to disease stage.

Nicholson WJ, Shepherd AJ, Aw DW.

Department of Medicial Microbiology, University Medical School, The University of Edinburgh, Scotland, UK.

This article reports on the development of PCR as a sensitive method of detecting both linear and circular forms of HIV-1 unintegrated viral DNA (UVD). The method was developed in a cell line study designed to follow the sequential synthesis of these forms over time. In all T lymphoid lineage cell lines, the full-length linear UVD (LUVD) was synthesized prior to both 1 and 2 LTR forms of circular UVD (CUVD), although all forms were detected by 12 hr postinoculation. Analysis of unstimulated PBMC samples from HIV-positive patients showed a significant difference in the presence of detectable CUVD forms and CDC groups II and IV ($p < 0.001$) and CDC groups III and IV ($p < 0.001$). No significance was demonstrated between CDC groups II and III ($p > 0.5$), linking the presence of CUVD forms to clinical disease and immunodeficiency. We propose that circular unintegrated forms of HIV-1 DNA may play a role in the development of acquired immunodeficiency syndrome.

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